REMARKS

The title is amended to more specifically reflect the elected subject matter. The specification is amended to update the cross-reference to related applications, and to provide generic terminology for various trademarks.

Claims 1-17 were previously pending.

Claims 7 and 10-17 are cancelled herein as directed to non-elected subject matter.

The status of Claims 2 and 4 is "Withdrawn" to indicate that they are not currently under examination, as they are directed to unelected species.

Claims 1, 3, 5, 6, 8 and 9 are rejected.

Claims 1, 5 and 8 are amended to recite the elected invention.

Claim 1 is further amended to recite that the antagonist of DCRS5 is either a binding composition derived from the antigen binding site of an antibody, or is a nucleic acid. Support for the amendment is found, e.g., at original Claims 5 and 8, and paragraph [0022] at page 7.

Applicants believe that no new matter is added by way of amendment.

I. Priority

The Examiner requested that the priority information in the first sentence of the specification be updated. The specification is amended herein to reflect the fact that that the Application No. 09/853,180 has issued as U.S. Patent No. 6,756,481.

The Examiner rejected priority to U.S. Provisional Patent Application No. 60/203,426 (the '426 application) on the grounds that that application allegedly did not include disclosure of "therapeutic methods using antagonist of DCRS5." To the contrary, therapeutic methods using antagonists of DCRS5 are disclosed in the '426 application.

Support is found, for example, at the following passages of the '426 application:

1) Pages 8-9 ("Therapeutic uses include methods of modulating physiology or development of a cell comprising contacting the cell with . . . an antagonist of p40/IL-B30 which is an antibody which binds a complex comprising: primate DCRS5 and/or primate IL-12Rβ1; an antagonist of p40/IL-B30 which is an antibody which binds to to

[SIC] DCRS5; . . . an antagonist of p40/IL-B30 which is an antisense nucleic acid to DCRS5 or IL-12Rβ1 . . . .").

2) Claims 21-22.

3) In the "Therapeutic Utility" section (page 57) antagonists are proposed for use in such diseases as autoimmunity, multiple sclerosis, psoriasis, chronic inflammatory conditions, rheumatoid arthritis, osteoarthritis or inflammatory bowel diseases. Such antagonists are proposed to take the form of "antibodies against the receptor subunits, soluble receptor constructs, or antisense nucleic acids to one or more of the receptor subunits." '426 Application at page 57. Antagonists are also proposed to "be useful as inhibitors of undesirable immune or inflammatory responses . . . ." '426 Application at page 58.

It is understood by one of skill in the art that antagonists of a ligand (p40/IL-B30) are also antagonists of its cognate receptor (DCRS5/IL-12Rβ1), since in each case the antagonist blocks signaling through the relevant pathway (IL-23/IL-23 receptor). *See. e.g.,* '426 Application at page 22 ("The terms ligand, agonist, antagonist, and analog of, e.g., a DCRS5, include molecules that modulate the characteristic cellular responses to cytokine ligand proteins, e.g. p40/IL-B30 . . . ."); pages 36-37 ("The blocking of physiological response to the receptor ligands may result from the inhibition of binding of the ligand to receptor, likely through competitive inhibition."); page 46 ("The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be potent antagonists that bind to the receptor and inhibit binding to ligand or inhibit the ability of the receptor to elicit a biological response . . . ."); page 11 ("Additionally, the invention provides matching of the p40/IL-B30 ligand with receptor subunits DCRS5 and IL-12Rβ1, which pairing provides insight into indications for use of agonists and antagonists based upon reagents directed thereto.").

In light of the disclosure of the use of antagonists of DCRS5 in therapeutic methods in the '426 Application, Applicants respectfully request that the present application be afforded priority to the filing date of the '426 Application (5/10/2000).[1]

[1] The Office Action includes a typographical error as to the filing date of the '426 Application.

II. Objections to the Specification

The Examiner requested that certain trademarks be presented in capitalized form and accompanied with generic terminology.

Applicants note that the trademark Taqplus® is provided in capitalized form, with a registered trademark symbol, and with accompanying generic terminology ("DNA polymerase"), and thus Applicants respectfully request withdrawal of the objection with respect to Taqplus®.

The specification is otherwise amended to comply with this request by adding generic terminology.

The Examiner further requested that paragraph 14 (page 4) be amended to insert β in place of a spurious symbol, which amendment is also included herein.

In view of the foregoing amendments, Applicants respectfully request withdrawal of the objections to the Specification.

III. Objections to the Claims

The Examiner objected to Claims 1 and 5 as including unelected inventions. These claims are amended herein to be directed to the elected invention.

The Examiner further indicated that Claims 2 and 4 would "not be examined further because they are drawn to unelected inventions" (species). The status of Claims 2 and 4 is updated with "Withdrawn" to indicate that these claims are directed to unelected species.

Currently pending Claims 1, 3, 5, 6, 8 and 9 read on the elected invention and species.

In view of the foregoing amendments, Applicants respectfully request withdrawal of the objections to the Claims.

IV. Rejection of Claims 1, 3, 5, 6, 8 and 9 under 35 U.S.C. §112, Second Paragraph

The Examiner rejected Claims 1, 3, 5, 6, 8 and 9 under 35 U.S.C. §112, second paragraph, because Claim 1 is allegedly vague and indefinite for use of the term "antagonist." It is alleged that there is no definition of antagonist in the specification,

that it is unclear which compounds could function as antagonists, and that one of skill in the art would not be able to determine which compounds could act as antagonists of DCRS5. Office Action at 4.

To the contrary, the specification is replete with specific examples of compounds that may serve as antagonists of DCRS5. For example, paragraph [0133] states that "Antagonists may take the form of antibodies against the receptor subunits, soluble receptor constructs,[2] antisense nucleic acids, or RNA interference nucleic acids, to one or more of the receptor subunits." Various forms of antagonists are also provided throughout the specification. Such exemplary antagonists are also provided at original Claims 5, 6, 8 and 9.

Nonetheless, solely to expedite prosecution, Applicants amend the claims herein to specify that antagonist refers only to the elected embodiments, *i.e.* a binding composition derived from the antigen binding site of an antibody, or a nucleic acid. In light of this amendment, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

V. Rejection of Claims 1, 3, 5, 6, 8 and 9 under 35 U.S.C. §112, First Paragraph

The Examiner rejected Claims 1, 3, 5, 6, 8 and 9 under 35 U.S.C. §112, first paragraph, for lack of **enablement** with respect to treatment of inflammatory bowel disease (IBD) with antagonists of DCRS5. Applicants respectfully disagree.

The specification discloses not only the biological activities of the newly discovered DCRS5 and the newly identified complex DCRS5/IL-12Rβ1 - it also discloses matching of this newly discovered heterodimeric cytokine receptor with its cognate ligand, IL-23 (p40/IL-B30). The matching of the IL-23 cytokine with its receptor is a crucial aspect of the present invention. Information relating to the known biological activities of the cytokine is necessarily very relevant to the receptor, since both the cytokine and receptor are, by definition, targets in the same regulatory pathway. This linking of DCRS5 ("IL-23R") to the IL-23 signaling pathway, in combination with the known activities of IL-23, would indicate to one of skill in the art that the DCRS5 polypeptide, nucleic acids encoding the polypeptide, and agonists and antagonists

[2] Soluble receptor is not a relevant antagonist in the currently pending claims, as-elected.

(such as antibodies) would find use in, e.g., diagnosis or treatment of chronic inflammatory disorders and autoimmune diseases.

This connection between the biological activity of DCRS5 (as a component of IL-23 receptor) and the known biological activity of IL-23 is expressed repeatedly in the specification, for example, at:

A) Page 3, paragraph [0010] (". . . the invention provides matching of the p40/IL-B30 ligand with receptor subunits DCRS5 and IL-12Rβ1, which pairing provides insight into indications for use of the agonists and antagonists based upon reagents directed thereto");

B) Page 16, paragraph [0045] ("The functional linkage of the receptor with the p40/IL-B30 ligand provides important insights into the clinical indications that the receptor will be useful for. Thus, antagonists and agonists will have predicted functional effects."); and

C) Page 34, paragraph [0105] ("The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be potent antagonists that bind to the receptor and inhibit binding to ligand . . ."); and

D) Page 38, paragraph [0118] ("Moreover, antibodies against the receptor subunits may serve to sterically block ligand binding to the functional receptor. Such antibodies may be raised to either subunit alone, or to the combination of DCRS5 with IL-12Rβ1. Antibody antagonists would result.").

E) Page 42, paragraph [0132], and page 49, paragraph [0154], where PCT Patent Application Publication WO 01/18051 is cited.[3] WO 01/18051 discloses the structure of the cytokine now known as IL-23, comprised of the IL-B30 ("p19") and IL-12p40 subunits. Antagonists of IL-23 are proposed to be useful in "autoimmune situations (such as multiple sclerosis or psoriasis) or chronic inflammatory conditions (such as rheumatoid arthritis or inflammatory bowel disease.)" WO 01/18051 at page 31, lines 9-12. IL-23 is proposed to play a role in "inflammation and/or autoimmune disorders," in which antagonists (such as blocking antibodies) may provide a powerful way to block these immune responses. WO 01/18051 at page 31, lines 9-12.

[3] See general statement of incorporation-by-reference at page 9, paragraph [0025].

The present specification indicates that antagonists of DCRS5 (IL-23R) would find use in treatment of IBD. *See, e.g.*, page 6, paragraph [0021]; page 18, paragraph [0052]; page 42, paragraph [0133]; page 46, paragraph [0144]; and at original Claims 1(e) and 3.

Post-filing publications have confirmed that antagonists of IL-23 (and thus its receptor DCRS5/IL-23R) are likely to have therapeutic utility in the treatment of IBD. Yen *et al*. found that IL-23 was involved in the production of a unique T cell subset (Th17 cells) that was responsible for chronic intestinal inflammation in a mouse model of human IBD. Yen *et al*. (2006) *J. Clin. Invest*. 116:1310 (attached). These authors showed that elimination of IL-23 ($p19^{-/-}$ mice - p19 knockouts) resulted in a dramatic reduction in chronic intestinal inflammation in mice that otherwise exhibit such inflammation. *Id.* at 1311, Figure 1. They also observed that blockade of the primary pro-inflammatory cytokines produced by these Th17 cells (IL-6 and IL-17) significantly reduced inflammation, suggesting that is was these IL-23 driven cells that were responsible for chronic inflammation. *Id.* at 1313, Figure 6. They also observed that administration of exogenous IL-23 accelerated the onset of intestinal inflammation in a T cell transfer model of colitis (*Id.* at 1310-1311), again suggesting that antagonists of IL-23 signaling may be useful as therapeutics for IBD.

Recently, Duerr *et al.* found that the gene encoding IL-23R (IL23R) was associated with IBD in a genome-wide association study. Duerr *et al.* (2006) *Science* 314:1461 (originally published in *Science Express* on 26-Oct-2006) (attached). Specifically, they found that a specific mutation (Arg381Gln) provided complete protection from IBD. The authors conclude that "[t]hese results and previous studies on the pro-inflammatory role of IL-23 prioritize this signaling pathway as a therapeutic target in inflammatory bowel disease." *Id.* at Abstract. One of skill in the art would understand that if a genetic knock-out of a gene provides protection from a disease (in this case IBD), antagonists of that gene or pathway would be expected to have similar beneficial effects. *Id.* at 1463 (". . . blockade of the IL-23 signaling pathway would be a rational therapeutic strategy for IBD.")

In light of the express relationship between the heterodimeric cytokine p40/IL-B30 (now known as IL-23) and the newly discovered cognate receptor complex DCRS5/IL-12RB1, and the known biological activity of IL-23 (cited and incorporated by reference), one of skill in the art would understand that antagonists of DCRS5 would find similar use as antagonists of IL-23. Because such uses included treatment of inflammatory bowel disease, the present specification adequately discloses and enables methods of treatment.

The Office Action goes on to point out the alleged lack of specificity with respect to various variables, such as half-life of therapeutic agents, proving a lack of adverse side effects, whether such an agent would penetrate to a target tissue. Such variables, however, cannot be fully evaluated in human subjects in anything short of a clinical trial. An Applicant for a patent is not required to provide results from human clinical trials. *See* MPEP 2107.03(III) ("In no case has a Federal Court required an applicant to support an asserted utility with data from human clinical trials.") In addition, such variables must be evaluated in the development of <u>any</u> potential human therapeutic agent and thus the necessary experimentation cannot be considered "undue" under the circumstances.

Accordingly, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112, first paragraph, for lack of enablement with respect to treatment of IBD with antagonists of DCRS5.

The Examiner further rejected Claims 1, 3, 5, 6, 8 and 9 under 35 U.S.C. §112, first paragraph, for lack of **enablement** and **written description** with respect to use of all possible antagonists of DCRS5 in the methods of the present invention. Although Applicants believe that the specification as-filed discloses a sufficient number of such antagonists to support a claim to antagonists generally,[4] solely in order to facilitate prosecution, the claims are amended herein to specify that "antagonist" refers to a binding composition derived from the antigen binding site of an antibody, or a nucleic

[4] Support is found for such DCRS5 antagonists as antibodies, antibody fragments, antisense [0135], and RNA interference (siRNA) nucleic acids [0135]. With regard to the nucleic acid-based antagonists, SEQ ID NO: 1 provides full written description support for sequences that would hybridize to nucleic acids encoding DCRS5, and thus further enables such embodiments.

acid. The claims as amended no longer relate to "all possible antagonists," and instead relate to specific species of antagonists, each of which is supported in the specification.

In light of these amendments to the claims, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112, first paragraph, for lack of enablement and written description with respect to the term "antagonists."

Conclusion

Applicants' current response is believed to be a complete reply to all the outstanding issues of the latest Office action. Further, the present response is a bona fide effort to place the application in condition for allowance or in better form for appeal. Accordingly, Applicants respectfully request reconsideration and passage of the amended claims to allowance at the earliest possible convenience.

Applicant believes that no additional fees are due with this communication. Should this not be the case, the Commissioner is hereby authorized to debit any charges or refund any overpayments to DNAX Deposit Account No. 04-1239.

If the Examiner believes that a telephonic conference would aid the prosecution of this case in any way, please call the undersigned.

Respectfully submitted,

Date: 29 May 2007

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Attachment 1: Yen *et al.* (2006) *J. Clin. Invest.* 116:1310

Attachment 2: Duerr *et al.* (2006) *Science* 314:1461

IL-23 is essential for T cell–mediated colitis and promotes inflammation via IL-17 and IL-6

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Uncontrolled mucosal immunity in the gastrointestinal tract of humans results in chronic inflammatory bowel disease (IBD), such as Crohn disease and ulcerative colitis. In early clinical trials as well as in animal models, IL-12 has been implicated as a major mediator of these diseases based on the ability of anti-p40 mAb treatment to reverse intestinal inflammation. The cytokine IL-23 shares the same p40 subunit with IL-12, and the anti-p40 mAbs used in human and mouse IBD studies neutralized the activities of both IL-12 and IL-23. *IL-10*–deficient mice spontaneously develop enterocolitis. To determine how IL-23 contributes to intestinal inflammation, we studied the disease susceptibility in the absence of either IL-23 or IL-12 in this model, as well as the ability of recombinant IL-23 to exacerbate IBD induced by T cell transfer. Our study shows that in these models, IL-23 is essential for manifestation of chronic intestinal inflammation, whereas IL-12 is not. A critical target of IL-23 is a unique subset of tissue-homing memory T cells, which are specifically activated by IL-23 to produce the proinflammatory mediators IL-17 and IL-6. This pathway may be responsible for chronic intestinal inflammation as well as other chronic autoimmune inflammatory diseases.

Introduction

When mucosal immunity is not countered by antiinflammatory mediators (e.g., IL-10 or TGF-β), excessive proinflammatory responses result in chronic inflammatory bowel disease (IBD) (1). To investigate the mechanism(s) responsible for IBD, we have studied 2 murine models: (a) *IL-10*–KO mice, which spontaneously develop enterocolitis (2) resembling Crohn disease and (b) lymphocyte-deficient Rag-KO mice, which develop colitis after reconstitution with $CD4^+$ T cells from *IL-10*–KO mice (3, 4). The intestinal disease that occurs in these models is initiated by the excessive generation of IFN-γ–producing T cells (Th1) driven by IL-12 produced by antigen-presenting cells. Thus, early treatment with anti–IFN-γ mAb (2, 4) or anti–IL-12(p40) mAb prevented disease (4). In contrast, treatment with anti–IL-12(p40) mAb, but not anti–IFN-γ mAb, reversed ongoing disease in both of our models (4) and in a chemically induced colitis model (5). Based on these outcomes, it was concluded that IL-12 might play a role in colitis independent of its ability to generate IFN-γ–producing T cells. However, recent studies have indicated that IL-23, which, like IL-12, is produced by antigen-presenting cells, and is also inhibited by anti-p40 mAb, regulates autoimmune-inflammatory processes in several mouse disease models (6–8). Support for a significant role of p40-containing cytokines (IL-12 and/or IL-23) in the pathogenesis of human disease comes from 2 early clinical trials (on Crohn disease and psoriasis) using p40-specific human mAbs (9, 10). In both trials, administration of anti-p40 resulted in improved clinical disease, but it was not clear whether this was due to neutralization of IL-12 or IL-23. To define the role of IL-12 versus IL-23 in chronic intestinal inflammation, we have conducted studies using *IL-10*–deficient mice to measure the individual contributions of these related cytokines.

Nonstandard abbreviations used: IBD, inflammatory bowel disease.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* 116:1310–1316 (2006). doi:10.1172/JCI21404.

Results

IL-12p35 × IL-10–KO but not IL-23p19 × IL-10–KO mice develop spontaneous IBD. To determine the relative contributions of IL-12 and IL-23 in IBD, we backcrossed IL-10–KO mice with mice lacking only *IL-12* ($p35^{-/-}$) or only *IL-23* ($p19^{-/-}$). As observed previously, *IL-10*–KO mice developed colitis by 3 monrhs of age (2). At 12 months, half of the *IL-10*–KO colony had wasted and died, and 100% of the survivors exhibited severe colitis (Figure 1, A and lower panel of C). Similarly to the *IL-10*–deficient animals, *IL-12p35* × *IL-10*–KO mice developed signs of colitis, including diarrhea and rectal prolapse as early as 7 weeks of age. Histological evaluation of colons from these animals at 3 months of age revealed marked intestinal inflammation, ruling out that IL-12 is required for the development of spontaneous IBD in *IL-10*–deficient animals (Figure 1B). However, when *IL-10*–KO mice were backcrossed with *p19*–KO mice, the *IL-10* × *p19*–double-KO mice were still disease free at 12 months of age (Figure 1, A and upper panel of C). This result suggested that IL-23, but not IL-12, is required for the manifestation of chronic intestinal inflammation. $CD4^+$ T cells from *IL-10* × *p19*–KO mice still produced large amounts of IFN-γ, indicating that *IL-10* × *p19*–KO mice are not impaired in their ability to generate a Th1 cell response (Figure 2A). The level of IFN-γ produced by $CD4^+$ T cells from *IL-10* × *p19*–KO mice consistently appeared increased over that of the *IL-10*–KO $CD4^+$ T cells, suggesting an enhanced Th1 response in *IL-10* × *p19*–KO mice. In contrast, $CD4^+$ T cells from *IL-10* × *p35*–KO mice showed reduced levels of IFN-γ (Figure 2B) Similar results were recently seen in the pathogenesis of 2 other chronic inflammatory autoimmune diseases, EAE and collagen-induced arthritis (CIA). In both diseases, Th1 responses developed normally in the absence of IL-23, but disease manifestation required the presence of IL-23.

Recombinant IL-23 accelerates T cell transfer colitis. To assess how IL-23 may be enhancing IBD, we performed studies in a T cell transfer model of colitis. The recipients of passively transferred T cells

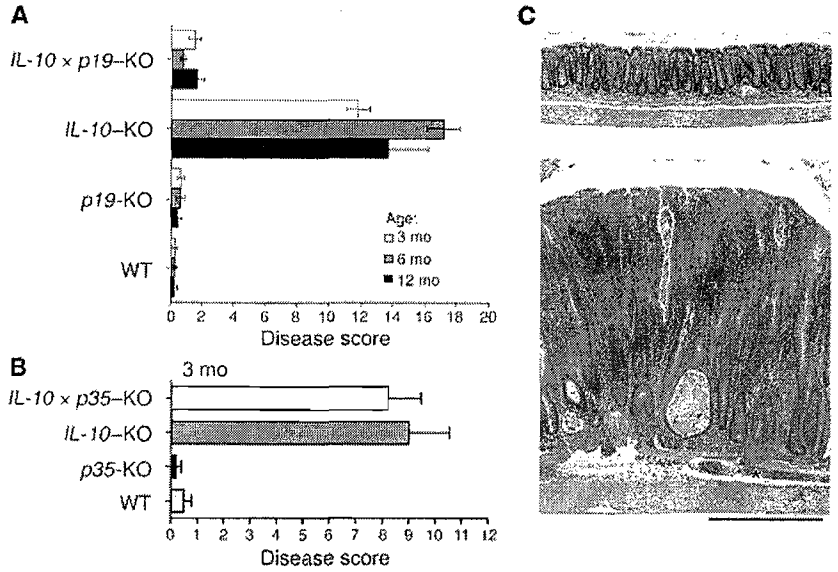

Figure 1
IL-23, but not IL-12, is essential for spontaneous colitis induced by IL-10 deficiency. (**A**) Histologic changes were evaluated at 3 time points in the colons of mice that were *IL-10* deficient and/or *IL-23* (*p19*) deficient. (**B**) Histologic changes were evaluated at 3 months in the colons of mice that were *IL-10* deficient and/or *IL-12* (*p35*) deficient. The disease scores for each group (n = 5–8) were obtained as previously described (41). (**C**) Photomicrographs of the descending colons from 12-month-old *IL-10* × *p19*–KO mice (upper panel) and *IL-10*–KO mice (lower panel). The lower panel shows marked mucosal thickening and epithelial hyperplasia. *Inflammation extended into the submucosa and tunica muscularis. Scale bar: 50 μm.

were *Rag*-KO mice, which are devoid of mature T and B cells. They normally develop colitis 10–12 weeks after reconstitution with either naive T cells ($CD4^+CD45RB^{high}$) or with memory T cells ($CD4^+CD45RB^{low}$) from diseased *IL-10*–KO mice (3, 4). However, recipients that were treated daily with IL-23 developed colitis after only 4 weeks (Figure 3A). The accelerated onset of colitis occurred regardless of whether IL-23–treated *Rag*-KO mice were reconstituted with naive or memory $CD4^+$ T cells. IL-23 treatment also led to splenomegaly and a blood neutrophilia (4,800 ± 800/mm³ of blood), whereas saline-treated controls still had normal spleens and baseline neutrophil counts (1,500 ± 200/mm³ of blood). The mesenteric LNs of IL-23–treated recipients contained greatly expanded numbers of $CD4^+$ T cells and $CD11b^-CDllc^+F4/80^-$ DCs (Figure 3B). Continuous infusions with IL-23 did not result in colitis in unreconstituted Rag-KO mice.

IL-23 promotes memory-activated T cell production of IL-6 and IL-17. In order to better define the actions of IL-23, gene expression studies were performed to investigate the mechanism by which IL-23 induces colitis using the T cell transfer model. For the gene expression analysis, gene-specific primers were used in TaqMan quantitative RT-PCR to detect the modulation of chemokines, cytokines, and cell-associated activation molecules. Gene expression of colon samples from recipients treated for 4 weeks with IL-23 (colitis) was compared with that of saline-treated controls (no colitis) and naive controls (no cell transfer). Many genes were upregulated following IL-23 treatment, which signified the influx of activated inflammatory macrophages (i.e., *IL-1β*, *TNF-α*, *NOS-2*) and granulocytes (i.e., myeloperoxidase, 12-lipoxygenase) (data not shown). In addition, increased expression of *monocyte chemoattractant protein–1* and *monokine induced by IFN-γ* (Figure 4A) as well as *MMP-7* and *MMP-12* (data not shown) may contribute to digestion of basement membranes and matrix proteins and the migration of cells into the mucosa. We also found that IL-23 treatment increased gene expression specific to T cells (CD3 ε chain; Figure 4A), confirming the rapid infiltration of colons by donor $CD4^+$ T cells. Early T cell engraftment was accompanied by increased gene expression for IFN-γ, which had already been linked to colitis in this model (2–4, 11). In addition, IL-23 treatment also resulted in increased IL-17 gene expression (Figure 4A).

Although IFN-γ can be made by accessory cells and T cells, IL-17 is predominantly made by T cells (12). Previous studies have shown that IL-17 is produced preferentially by human and murine T cells with a memory/activated phenotype (13, 14). To further examine the role of IL-23 in activation of memory T cells, sorted $CD4^+CD45RB^{low}$ memory T cells from *IL-10*–KO mice were assessed for cytokine gene expression following stimulation in the presence or absence of IL-23. As shown in Figure 4B, the mRNA levels of TNF and IFN-γ were unchanged by IL-23. In contrast, IL-17 gene expression was elevated by IL-23. In parallel experiments (data not shown), it was found that IL-23 had no effect on gene expression by naive T cells, consistent with their failure to express the IL-23 receptor (15). In addition to elevation of IL-17 gene expression, IL-23 also strongly induced gene expression of IL-6 (Figure 4B).

Memory T cells from WT and mutant mice were then assayed for cytokine secretion using ELISA. In the presence of plate-bound anti-CD3 mAb, IL-23 specifically stimulated increased IL-17 production (Figure 5A). The highest level of IL-17 was induced by IL-23 in cells from *IL-10*–KO mice. IL-17 was also produced by cells from *IL-10* × *p19*–KO mice but was very low in cells from WT and *p19*-KO

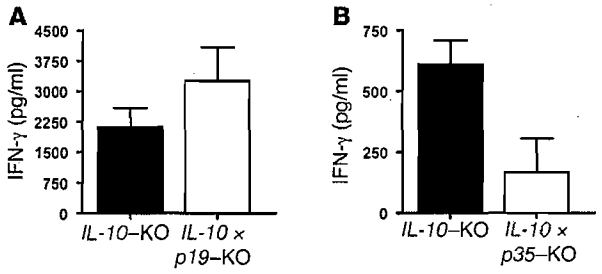

Figure 2
Normal production of IFN-γ in the absence of IL-23, but not IL-12. $CD4^+$ splenocytes from *IL-10*–deficient mice and mice doubly deficient for *IL-10* and *IL-23p19* (**A**) or *IL-12p35* (**B**) were stimulated with anti-CD3 and anti-CD28 for 3 days. Supernatants were assessed for IFN-γ as described in Methods. Bars represent 3 mice per group; error bars indicate SD. Data shown are representative of 2 experiments.

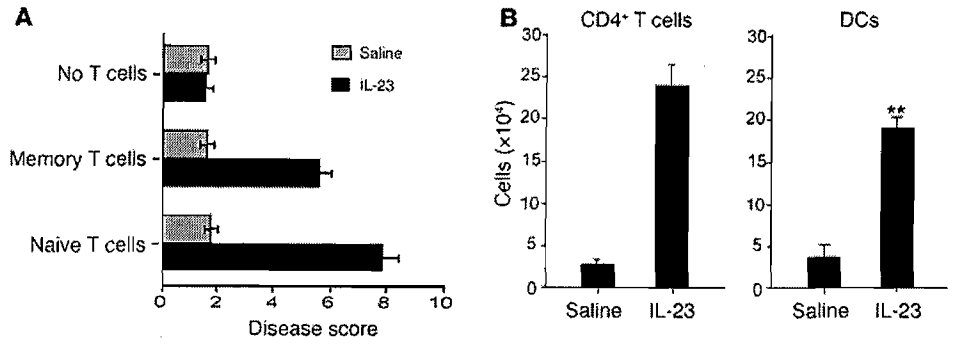

Figure 3
IL-23 treatment accelerates the onset of colitis in T cell–reconstituted immunodeficient recipients. (**A**) Disease scores of *Rag*-KO mice reconstituted with naive or memory CD4+ T cells from *IL-10*–KO donors followed by daily infusions with IL-23 (1 μg/dose) or saline for 4 weeks (n = 8). (**B**) T cell and accessory cell numbers in mesenteric lymph nodes of reconstituted *Rag*-KO mice treated with IL-23 or saline for 4 weeks.

mice. Basal levels of IL-4 and IFN-γ were not modulated by either IL-12 or IL-23 costimulation. When we assayed for IL-23–dependent proliferation in the presence of anti-IL-2 mAb, we found that memory cells from *IL-10*–KO were the best responders (Figure 5B). In contrast, memory T cells from all 4 strains proliferated equally in cultures supplemented with IL-2 (data not shown). Interestingly, there appeared to be a direct correlation between the ability of cells to produce IL-17 in response to IL-23 and the degree of IL-23–stimulated growth. To understand the correlation between IL-23–induced T cell development and IL-17 production, memory T cells were prepared for intracellular cytokine staining. Cells from *IL-10*–KO mice and *IL-10* × *p19*–KO mice contained distinct populations that could produce IL-4 or IFN-γ (Figure 5C). Strikingly, only diseased *IL-10*–KO mice had a large number of IL-17–positive cells. The vast majority of the IL-17–positive cells were negative for IL-4 and IFN-γ staining, showing that they constitute a subset distinct from classical Th1 and Th2 memory cells. The small number of IL-17–staining T cells present in *IL-10* × *p19*–KO mice (Figure 5C) suggests that the generation of this particular T cell subset can occur in the absence of endogenous IL-23 production. Data presented in Figure 5, A and B, indicate, however, that optimal expansion and IL-17 production require IL-23 stimulation.

Blockade of IL-6 and IL-17 ameliorates IBD. Our results suggest that IL-23 promotes production of IL-6 and IL-17 by memory activated T cells (Figures 4B and 5) from *IL-10*–KO mice with IBD. Many reports have shown that IL-6 plays a crucial role in the pathogenesis of chronic IBD (16–18), while the role of IL-17 remains unclear. IL-17 mRNA expression was upregulated in inflamed colons of *IL-10*–KO but not in the noninflamed colons of WT, *p19*-KO, and *IL-10* × *p19*–double-KO mice (data not shown).

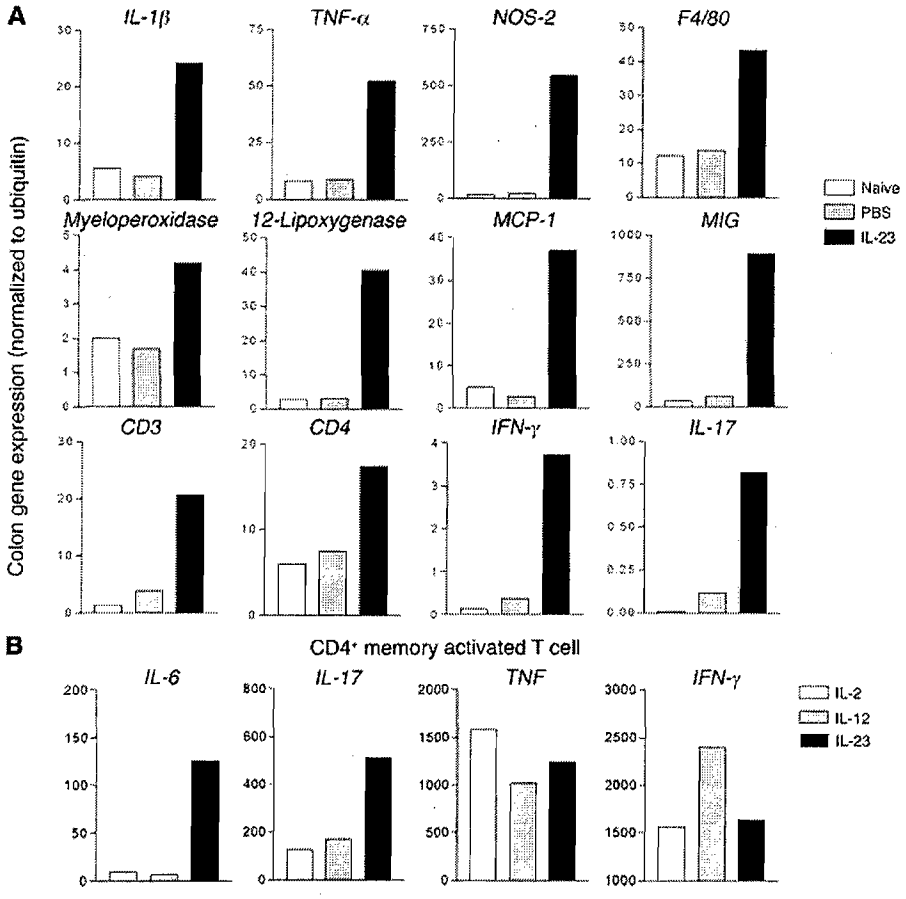

Figure 4
Gene expression induced by IL-23 treatment of *Rag*-KO mice reconstituted with memory CD4+ T cells from *IL-10*–KO mice. (**A**) Increased mRNA expression in colons of *Rag*-KO mice reconstituted with memory CD4+ T cells from *IL-10*–KO mice and treated with IL-23 for 4 weeks. mRNA from the colons of 4 mice were pooled, and gene expression levels were determined by real-time quantitative PCR. Data are presented as values normalized to ubiquitin. (**B**) Gene expression by memory CD4+ T cells from *IL-10*–KO mice following in vitro stimulation with anti-CD3 mAb plus IL-2, IL-12, or IL-23 relative to stimulation with anti-CD3 mAb alone.

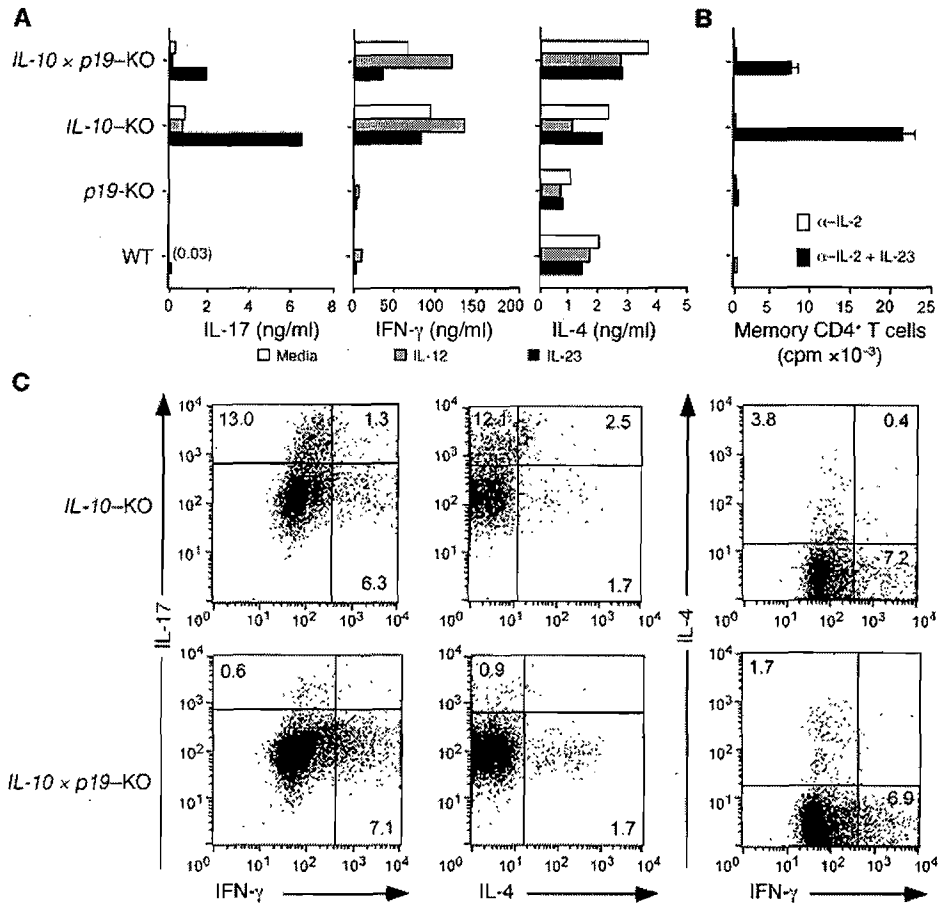

Figure 5
IL-23, but not IL-12, specifically stimulates a subset of memory CD4+ T cells that produce IL-17. FACS-purified CD4+CD45RB^{low} splenic T cells (2×10^5/ml; >95% purity) were isolated and cultured on CD3-coated plates. (**A**) Cytokine production by memory CD4+ T cells. Supernatants from cells stimulated with IL-23 (10 ng/ml) or IL-12 (1 ng/ml) were analyzed by ELISA. (**B**) IL-23–dependent proliferation of memory CD4+ T cells was assessed by[^{3}H]-thymidine incorporation after 4 days of culture on anti-CD3 mAb–coated plates in the presence of anti–IL-2 mAb. (**C**) FACS analysis of memory CD4+ T cells from *IL-10*–KO and *IL-10* × *p19*–KO mice after cells were stained for intracellular IL-4, IL-17, and IFN-γ proteins.

Heightened IL-6 (19) and IL-17 levels (20) could be detected in both serum and intestinal tissue from patients with Crohn disease. Blocking the IL-6 signaling pathway prevented the development of T cell–mediated murine colitis (16, 17). Furthermore, several potent proinflammatory factors such as IL-1, TNF, as well as IL-6 are downstream mediators of IL-17 (21). In order to address whether IL-23 induction of IL-6 and IL-17 production plays a role in the pathogenesis of bowel inflammation, we cotreated T cell–restored recipient mice with IL-23 as well as Abs that neutralize IL-6, IL-17, or both cytokines. Isotype control Ab-treated mice developed enterocolitis (path score = 10) 6 weeks after treatment, while the single Ab-treated groups (anti-IL-6: 6.5 and anti-IL-17: 6) developed an attenuated intestinal inflammation (Figure 6). More importantly, anti-IL-6 and anti-IL-17 combination therapy significantly ameliorated the severity of intestinal inflammation induced by IL-23. Taken together, these results suggest that IL-23 promotes development and expansion of a pathogenic IL-6/IL-17–producing memory-activated T cell population that can trigger the inflammatory cascade leading to intestinal inflammation.

Discussion

Our studies with IL-23–deficient mice show that IL-23 is essential for the manifestation of intestinal inflammation. This is consistent with the recently described dominant role for IL-23 in both CNS and joint autoimmune inflammation (6, 7). Together these findings point to IL-23, but not IL-12, as the necessary mediator of organ-specific autoimmune diseases. Although IL-23 and IL-12 share structural homologies, they have very distinct biological activities. The first striking difference between IL-23 and IL-12 is that IL-23 specifically stimulates memory CD4+ T cells, whereas IL-12 is a potent stimulant for naive CD4+ T cells (22, 23). The selective activation of memory T cells by IL-23 is especially relevant to tissue inflammation. Memory T cells are well known for their tissue-homing properties. In the case of our IBD models, memory CD4+ T cells constitute 60–80% of the T cells found in the intestines of mice with colitis (3). Furthermore, reconstitution of immunodeficient SCID or *Rag*-KO mice with naive CD4+ T cells rapidly leads to the selective expansion and intestinal engraftment of donor T cells expressing a memory phenotype (24). Lamina propria DCs, particularly in the distal end of the small intestine and driven by the intestinal flora, were recently described as constitutively expressing IL-23, suggesting a predisposition of this part of the small intestine to initiate chronic inflammatory responses through IL-23 (25). Interestingly, the constitutive expression of IL-23 in this part of the small intestine was accompanied by a peak in IL-17 expression.

A second important difference is that IL-23, unlike IL-12, induces the production of IL-17 by a unique subset of memory T cells. Therefore, IL-23 regulates a highly potent T cell–derived cytokine that has broad actions on the immune system. IL-17 is known to stimulate fibroblasts, endothelial cells, macrophages, and epithelial cells to secrete multiple proinflammatory mediators (i.e.,

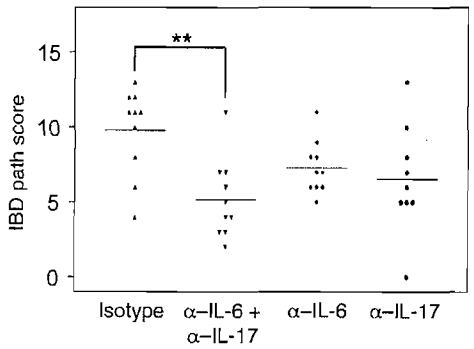

Figure 6

Blocking IL-6 and IL-17 significantly reduced the intestinal inflammation, by 50%. Recipient mice were dosed i.p. with isotype, anti–IL-6, anti–IL-17, or anti–IL-6 plus anti–IL-17 Abs (2 mg/mouse) a day prior to T cell reconstitution. *Rag*-KO mice were reconstituted with sorted splenic $CD4^+CD45RB^{hi}$ (naive) T cells (5×10^5 cells/mouse) from diseased *IL-10*–KO mice and treated daily with 1 μg/mouse IL-23 protein. Subsequent rounds of Ab were administered weekly for 6 weeks. The graph shows the path scores from 2 independent but identical experiments. The disease scores for each group were obtained as previously described (41). Horizontal bars represent the median value for each group. $^{**}P < 0.05$, compared with isotype Ab (unpaired Student's *t* test). Histologic examination was performed and scored using formalin-fixed tissue sections stained with H&E, as previously described (40).

IL-1, IL-6, TNF, NOS-2, metalloproteases, and chemokines) (14). Importantly, in vivo studies have indicated that the local production of IL-17 may cause a site-specific influx and activation of inflammatory cells. In mice, IL-17 administered into the peritoneal cavity elicited peritonitis (26), and intratracheal administration elicited lung inflammation (27, 28). IL-17 has been identified in the synovial fluids of patients with rheumatoid arthritis (29, 30) and Lyme disease (31). T cells isolated from the inflamed joints of patients with Lyme disease contained a subset of IL-17–producing T cells that were distinct from those producing IL-4 or IFN-γ. This pattern is similar to what we observed with memory T cells isolated from mice with colitis. It is also similar to the observations by Murphy et al. in a model of collagen-induced arthritis using IL-23–deficient mice (7). Resistance to the development of joint and bone pathology in these mice correlated with an absence of IL-17–producing $CD4^+$ T cells despite normal induction of collagen-specific, IFN-γ–producing Th1 cells. In separate studies, it was shown that either neutralizing Th1 responses by anti-p40 mAb (32–34) or blocking IL-17 (35) led to diminished chronic joint inflammation in mice. The connection between these 2 successful treatments was not understood, since IL-17 is not associated with "classical" Th1 cytokine responses (36). Given the data presented here, it is not surprising that both treatments were protective. A similar unique CD4 T cell activation state characterized by the production of IL-17 in response to IL-23 has recently been observed by Aggarwal et al. (37). Our results show that $CD4^+$ T cells from *IL-10* × *p19*-KO mice still produce high levels of IFN-γ. If anything, the level of IFN-γ produced by cells from these mice appears to be somewhat higher, suggesting an increased Th1 response in these animals in the absence of IL-23, although other Th1 cytokines were largely unchanged. In contrast, $CD4^+$ T cells from *IL-10* × *p35*-KO mice showed reduced production of IFN-γ. Since the production of IFN-γ is highly dependent on the presence of IL-12, this result was not unexpected. However, despite the reduced level of IFN-γ, these animals develop colitis very early, while the *IL-10* × *p19*-KO mice remain resistant to colitis development.

Since our studies were conducted in *IL-10*–deficient mice and employed cells in the transfer colitis model obtained from *IL-10*–deficient mice, one could question whether similar results would be obtained in mice with normal IL-10 levels. The absence of IL-10–related regulatory effects could account for the increased disease proclivity in these mice. In this context, it is interesting to note that there was increased IL-17 production in *IL-10*–deficient mice as compared with WT mice (Figure 5A). It is possible that in the presence of normal IL-10 levels, IL-23–mediated inflammation alone would not be sufficient to support mucosal inflammation and would require additional inflammatory signals such as those mediated by IL-12. Several recent EAE and CIA studies, representing commonly used models of chronic inflammation and autoimmunity, have shown that mice deficient in IL-23, but with otherwise normal expression of IL-10, are protected from disease (6–8), making it less likely that our results are only relevant in the absence of IL-10.

Very recently, Langrish et al. (8) have identified a pathogenic IL-23–dependent T cell population that is essential for the establishment of organ-specific inflammation associated with autoimmunity. This $Th_{IL\text{-}17}$ population, characterized by the production of IL-17, IL-6, and TNF, can be distinguished from IL-12–driven T cells with respect to the expression pattern of proinflammatory cytokines and other factors. Furthermore, transfer studies showed that these IL-23–dependent $Th_{IL\text{-}17}$ $CD4^+$ T cells, but not IL-12–dependent Th1 $CD4^+$ T cells, are essential for the establishment of inflammation associated with CNS autoimmunity. The data presented here are strikingly similar. $CD4^+CD45RB^{low}$ memory T cells isolated from *IL-10*–KO mice produce IL-6 and IL-17 following stimulation in the presence of IL-23. These cells also produce TNF, but this cytokine is induced in response to both IL-23 and IL-12, suggesting that TNF may play a role in both IL-12– and IL-23–linked immune responses. A similar result was obtained after in vitro stimulation of $Th_{IL\text{-}17}$ and Th1 $CD4^+$ T cells (8). In addition to IL-17, the production of IL-6 by these $CD4^+CD45RB^{low}$ memory T cells is also uniquely dependent on the presence of IL-23. Langrish et al. (8) speculate that IL-6 produced by the pathogenic $Th_{IL\text{-}17}$ $CD4^+$ T cells may in fact inhibit the action of regulatory T cell function, allowing effector T cell activation (38). Although the data presented here do not address that question, it is evident that neutralization of IL-6 has a beneficial effect on the IL-23–induced intestinal inflammation. The significant amelioration of disease observed with combined anti–IL-6 and anti–IL-17 treatment demonstrates that the action of IL-6 and IL-17, both produced by this pathogenic T cell population, is not redundant and that these 2 inflammatory mediators are to a large extent responsible for the IL-23–driven intestinal inflammation. It is interesting to note that a small number of IL-17–staining T cells remain in *IL-10* × *p19*-KO mice. Similarly, Aggarwal et al. also noted that not all IL-17 production is abrogated in the absence of IL-23 (37).

Although we find that memory $CD4^+$ T cells are a critical target of IL-23 in the development of chronic intestinal inflammation, it is likely not the only cell population that responds to IL-23. In CNS autoimmune inflammation, IL-23 was found to act more broadly as an end-stage effector cytokine through direct actions on macrophages in addition to its effect on memory $CD4^+$ T cells (6, 7). Other studies have identified DCs as a potential target cell

population for IL-23 (39). The relative contributions of these cell subsets with respect to IL-23 and disease pathogenesis have not yet been determined.

Our data provide evidence that the activation of tissue-homing memory T cells by IL-23 is responsible for chronic intestinal inflammatory disease. We show that this cytokine induces a distinct T cell activation state that is characterized by the production of several proinflammatory cytokines, including IL-17 and IL-6, but not IFN-γ. A similar IL-23–responsive, IL-17/IL-6/TNF-producing activated memory T cell subset is essential in the pathogenesis of other chronic autoimmune diseases, highlighting the importance of this novel pathway in chronic autoimmune inflammatory disease. This is even more relevant, since recent clinical trials demonstrate improved clinical disease after treatment with anti-p40 Abs (9, 10). Since these anti-p40 Abs neutralize both IL-12 and IL-23, it is not clear whether the therapeutic effects are due to neutralization of IL-12 or IL-23. Our data identify IL-23 as the relevant target in colitis.

Methods

Experimental mice. IL-10-KO mice and littermate *IL-10* × *p19*-KO, *p19*-KO (6), *IL-10* × *p35*-KO, and WT mice on the C57BL/6 × 129/SvEv background were generated and maintained at DNAX. C57BL/6 mice, Rag-KO mice, and *IL-12* (*p40*)-KO mice on the BALB/c mouse background as well as *IL-12* (*p40*)- and *IL-12* (*p35*)-KO mice on the C57BL/6 background were purchased from the Jackson Laboratory. *Rag*-KO 129/SvEv, *IL-10*-KO 129/SvEv, and *IL-10*-KO BALB/c mice were bred at DNAX. All animal procedures were approved by the Schering-Plough Biopharma Institutional Animal Care and Use Committee committee, in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International.

Histopathology. Histologic examination of colons from IBD experiments was performed by a veterinary pathologist using formalin-fixed tissue sections stained with H&E, as previously described (40).

T cell reconstitution of Rag-KO mice. Splenic T cells from *IL-10*-KO donors were enriched by red cell lysis and magnetic bead depletion using lineage-specific mAb supernatants B220 (B cells), 8C5 (neutrophils), Mac-1 (macrophages), Ter 119 (erythrocytes), and anti-CD8 (BD Biosciences – Pharmingen). Stained cells were removed in a magnetic field using goat anti-rat IgG (Fc)- and anti-rat IgG (H + L)-coated magnetic beads (Perseptive Diagnostics). The remaining cells were stained with CD4-PE and CD45RB-FITC (BD Biosciences – Pharmingen) for sorting on a FACSVantage SE (BD). 5×10^5 $CD4^+CD45RB^{high}$ (naive) T cells or $CD4^+CD45RB^{low}$ (memory) T cells were injected i.p. into *Rag*-KO recipients. Recipients were injected i.p. daily with 1 μg purified mouse IL-23 (22).

Cytokine and proliferation assays. FACS-purified $CD4^+CD45RB^{low}$ T cells from the spleen (2×10^5/ml; >95% purity) were cultured on CD3-coated plates in medium supplemented with 50 ng/ml IL-12 (R&D Systems) or 50 ng/ml IL-23. Supernatants were collected after 96 hours and assayed for IL-4, IFN-γ (Ab pairs from BD Biosciences – Pharmingen), and IL-17 by ELISA kit (R&D Systems) according to the manufacture's directions. For IFN-γ assay, $CD4^+$ splenocytes were purified using CD4-specific magnetic beads on an automated magnetic cell sorter (Miltenyi Biotec) following the manufacturer's instructions. Cells were cultured for 72 hours with 1 μg/ml anti-CD28 (BD Biosciences – Pharmingen) on anti-CD3-coated plates. IFN-γ was assessed in supernatants by fluorescent bead array using IFN-γ-specific beads (Upstate USA Inc.) on a Luminex 100 analyzer instrument (Luminex Corp.) according to the manufacturer's recommendation. For proliferation assays, T cells were cultured on anti-CD3-coated plates supplemented with IL-2 or with IL-23 and neutralizing anti-IL-2 mAb. Cell cultures were pulsed after 96 hours with 1 μCi [^{3}H]thymidine per well for 24 hours. Incorporation was measured by liquid scintillation spectroscopy, and data are expressed as mean counts per minute of triplicate wells. DCs were obtained from spleens of *Rag*-KO mice by depleting macrophages (F4/80) and erythrocytes by magnetic bead depletion as described above. The remaining cells were stained with anti-I-A, anti-CDllc, and anti-Mac-1 mAbs and sorted to greater than 95% purity. Cells were cultured with LPS (10 μg/ml) and/or IL-23 (50 ng/ml). Cell supernatants were harvested after 72 hours and assayed by ELISA. For intracellular cytokine analysis, memory $CD4^+$ T cells were stimulated with PMA and ionomycin for 4 hours. Brefeldin A (EPICENTRE Biotechnologies) was adding during the last 2 hours of stimulation. Cells were harvested, fixed in 4% formaldehyde, permeabilized with 0.1% saponin, and then stained with FITC- or PE-conjugated anti-cytokine or isotype control mAb (BD Biosciences – Pharmingen). Cells were analyzed using a FACSCalibur and CellQuest software (BD).

RNA expression analysis. Total RNA was prepared from frozen colon tissue samples or from activated $CD4^+CD45RB^{low}$ T cells using RNeasy kit (QIAGEN) according to the manufacturer's protocols. Total RNA (5 μg) was treated with DNAse I (Ambion Inc.) to remove contaminating genomic DNA, then reverse transcribed with Superscript II reverse transcriptase (Invitrogen Corp.) with oligo $p(DT)_{15}$ (Roche Applied Science) and random hexamers (Promega), according to the manufacturer's protocol. Gene expression levels were determined by real-time quantitative PCR using an ABI 5700 sequence detector system (Applied Biosystems) and SYBR Green PCR Master Mix according to the manufacturer's instructions (Applied Biosystems). Primers were designed to ensure no cross-reactivity with other genes and, where possible, were designed to span intron/exon boundaries. PCR amplification of the housekeeping gene ubiquitin was performed for each sample to control for loading and to allow normalization between samples. Both water and genomic DNA controls were included to ensure specificity. Each data point was evaluated for integrity by analysis of the amplification plot and dissociation curves. Data normalized to ubiquitin were then compared and expressed as the fold induction of gene expression in treated samples versus control samples.

In vivo administration of mAbs. Rag-KO recipient mice were injected i.p. with 2 mg/mouse of isotype control (rat IgG1 GL113), anti-IL-17 (rat anti-mIL-17 IgG1 18H10), anti-IL-6 (rat anti-mIL-6 IgG1 MP5-20F3), or anti-IL-6 and anti-IL-17 combination mAb 1 day prior to naive *IL-10*-KO T cell reconstitution. Following T cell reconstitution, mice were dosed i.p. with 1 μg/mouse IL-23 protein daily and 2 mg/mouse total Ab weekly for 6 weeks. The health of mice was monitored daily to observe any gross signs of colitis (wasting and diarrhea).

Statistics. Unpaired Student's *t* test was used to analyze data. Differences were considered significant at $P < 0.05$.

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1. Braegger, C.P. 1994. Immunopathogenesis of chronic inflammatory bowel disease. *Acta Paediatr. Suppl.* **83**:18–21.
2. Berg, D.J., et al. 1996. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. *J. Clin. Invest.* **98**:1010–1020.
3. Davidson, N.J., et al. 1996. T helper cell 1-type CD4+ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. *J. Exp. Med.* **184**:241–251.
4. Davidson, N.J., et al. 1998. IL-12, but not IFN-gamma, plays a major role in sustaining the chronic phase of colitis in IL-10-deficient mice. *J. Immunol.* **161**:3143–3149.
5. Neurath, M.F., et al. 1995. Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J. Exp. Med.* **182**:1281–1290.
6. Cua, D.J., et al. 2003. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature.* **421**:744–748.
7. Murphy, C.A., et al. 2003. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. *J. Exp. Med.* **198**:1951–1957.
8. Langrish, C.L., et al. 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* **201**:233–240.
9. Mannon, P.J., et al. 2004. Anti-interleukin-12 antibody for active Crohn's disease. *N. Engl. J. Med.* **351**:2069–2079.
10. Kauffman, C.L., et al. 2004. A phase I study evaluating the safety, pharmacokinetics, and clinical response of a human IL-12 p40 antibody in subjects with plaque psoriasis. *J. Invest. Dermatol.* **123**:1037–1044.
11. Powrie, F., et al. 1994. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunity.* **1**:553–562.
12. Yao, Z., et al. 1995. Human IL-17: a novel cytokine derived from T cells. *J. Immunol.* **155**:5483–5486.
13. Shin, H.C., et al. 1999. Expression of IL-17 in human memory CD45RO+ T lymphocytes and its regulation by protein kinase A pathway. *Cytokine.* **11**:257–266.
14. Fossiez, F., et al. 1998. Interleukin-17. *Int. Rev. Immunol.* **16**:541–551.
15. Parham, C., et al. 2002. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *J. Immunol.* **168**:5699–5708.
16. Yamamoto, M., et al. 2000. IL-6 is required for the development of Th1 cell-mediated murine colitis. *J. Immunol.* **164**:4878–4882.
17. Atreya, R., et al. 2000. Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in Crohn disease and experimental colitis in vivo. *Nat. Med.* **6**:583–588.
18. Ito, H., et al. 2002. Anti-IL-6 receptor monoclonal antibody inhibits leukocyte recruitment and promotes T-cell apoptosis in a murine model of Crohn's disease. *J. Gastroenterol.* **37**(Suppl. 14):56–61.
19. Gross, V., et al. 1992. Evidence for continuous stimulation of interleukin-6 production in Crohn's disease. *Gastroenterology.* **102**:514–519.
20. Fujino, S., et al. 2003. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut.* **52**:65–70.
21. Hata, K., et al. 2002. IL-17 stimulates inflammatory responses via NF-kappaB and MAP kinase pathways in human colonic myofibroblasts. *Am. J. Physiol. Gastrointest. Liver Physiol.* **282**:G1035–G1044.
22. Oppmann, B., et al. 2000. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity.* **13**:715–725.
23. Trinchieri, G. 1998. Proinflammatory and immunoregulatory functions of interleukin-12. *Int. Rev. Immunol.* **16**:365–396.
24. Reimann, J., et al. 1993. Selective engraftment of memory CD4+ T cells with an unusual recirculation pattern and a diverse T cell receptor-V beta repertoire into scid mice. *Eur. J. Immunol.* **23**:350–356.
25. Becker, C., et al. 2003. Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells. *J. Clin. Invest.* **112**:693–706. doi:10.1172/JCI200317464.
26. Witowski, J., et al. 2000. IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GRO alpha chemokine from mesothelial cells. *J. Immunol.* **165**:5814–5821.
27. Linden, A., Hoshino, H., and Laan, M. 2000. Airway neutrophils and interleukin-17. *Eur. Respir. J.* **15**:973–977.
28. Laan, M., et al. 1999. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *J. Immunol.* **162**:2347–2352.
29. Chabaud, M., et al. 1999. Human interleukin-17: a T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. *Arthritis Rheum.* **42**:963–970.
30. Kotake, S., et al. 1999. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J. Clin. Invest.* **103**:1345–1352.
31. Infante-Duarte, C., et al. 2000. Microbial lipopeptides induce the production of IL-17 in Th cells. *J. Immunol.* **165**:6107–6115.
32. Malfait, A.M., et al. 1998. Blockade of IL-12 during the induction of collagen-induced arthritis (CIA) markedly attenuates the severity of the arthritis. *Clin. Exp. Immunol.* **111**:377–383.
33. Matthys, P., et al. 1998. Anti-IL-12 antibody prevents the development and progression of collagen-induced arthritis in IFN-gamma receptor-deficient mice. *Eur. J. Immunol.* **28**:2143–2151.
34. Joosten, L.A., et al. 1997. Dual role of IL-12 in early and late stages of murine collagen type II arthritis. *J. Immunol.* **159**:4094–4102.
35. Lubberts, E., et al. 2001. IL-1-independent role of IL-17 in synovial inflammation and joint destruction during collagen-induced arthritis. *J. Immunol.* **167**:1004–1013.
36. Lenarczyk, A., et al. 2000. Antigen-induced IL-17 response in the peripheral blood mononuclear cells (PBMC) of healthy controls. *Clin. Exp. Immunol.* **122**:41–48.
37. Aggarwal, S., et al. 2003. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J. Biol. Chem.* **278**:1910–1914.
38. Pasare, C., and Medzhitov, R. 2003. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. *Science.* **299**:1033–1036.
39. Belladonna, M.L., et al. 2002. IL-23 and IL-12 have overlapping, but distinct, effects on murine dendritic cells. *J. Immunol.* **168**:5448–5454.
40. Simpson, S.J., et al. 1998. T cell-mediated pathology in two models of experimental colitis depends predominantly on the interleukin 12/Signal transducer and activator of transcription (Stat)-4 pathway, but is not conditional on interferon gamma expression by T cells. *J. Exp. Med.* **187**:1225–1234.
41. Braegger, C.P. 1994. Immunopathogenesis of chronic inflammatory bowel disease. *Acta Paediatr. Suppl.* **83**:18–21.

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Figs. S1 to S13
References

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A Genome-Wide Association Study Identifies *IL23R* as an Inflammatory Bowel Disease Gene

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The inflammatory bowel diseases Crohn's disease and ulcerative colitis are common, chronic disorders that cause abdominal pain, diarrhea, and gastrointestinal bleeding. To identify genetic factors that might contribute to these disorders, we performed a genome-wide association study. We found a highly significant association between Crohn's disease and the *IL23R* gene on chromosome 1p31, which encodes a subunit of the receptor for the proinflammatory cytokine interleukin-23. An uncommon coding variant (rs11209026, c.1142G>A, p.Arg381Gln) confers strong protection against Crohn's disease, and additional noncoding *IL23R* variants are independently associated. Replication studies confirmed *IL23R* associations in independent cohorts of patients with Crohn's disease or ulcerative colitis. These results and previous studies on the proinflammatory role of IL-23 prioritize this signaling pathway as a therapeutic target in inflammatory bowel disease.

Crohn's disease (CD) and ulcerative colitis (UC), the two common forms of idiopathic inflammatory bowel disease (IBD), are chronic, relapsing inflammatory disorders of the gastrointestinal tract. Each has a peak age of onset in the second to fourth decades of life and prevalences in European ancestry populations that average about 100 to 150 per 100,000 (*1*, *2*). Although the precise etiology of IBD remains to be elucidated, a widely accepted hypothesis is that ubiquitous, commensal intestinal bacteria trigger an inappropriate, overactive, and ongoing mucosal immune response that mediates intestinal tissue damage in genetically susceptible individuals (*1*). Genetic factors play an important role in IBD pathogenesis, as evidenced by the increased rates of IBD in Ashkenazi Jews, familial aggregation of IBD, and increased concordance for IBD in monozygotic compared to dizygotic twin pairs (*3*). Moreover, genetic analyses have linked IBD to specific genetic variants, especially *CARD15* variants on chromosome 16q12 and the *IBD5* haplotype (spanning the organic cation transporters, *SLC22A4* and *SLC22A5*, and other genes) on chromosome 5q31 (*3*–*7*). CD and UC are thought to be related disorders that share some genetic susceptibility loci but differ at others.

The replicated associations between CD and variants in *CARD15* and the *IBD5* haplotype do not fully explain the genetic risk for CD, so we performed a genome-wide association study testing 308,332 autosomal single nucleotide polymorphisms (SNPs) on the Illumina HumanHap300 Genotyping BeadChip (*8*). Our study population consisted of 567 non-Jewish, European ancestry patients with ileal CD and 571 non-Jewish controls. We initially focused on ileal CD, the most common location of CD, to minimize pathogenic heterogeneity. After exclusion of study subjects with genotype completion rates less than 94%, we included 547 cases and 548 controls in subsequent analyses (*8*). Single-marker allelic tests were performed using χ^2 statistics for all autosomal markers. Three SNPs had nearly two orders of magnitude greater significance compared to the next most significant markers, and they are the only markers that remain significant at the 0.05 level after Bonferroni correction. Two of the three markers, rs2066843 ($P = 2.86 \times 10^{-9}$, corrected $P = 8.82 \times 10^{-4}$) and rs2076756 ($P = 5.12 \times 10^{-10}$, corrected $P = 1.58 \times 10^{-4}$), are in the known CD susceptibility gene, *CARD15* (*4*, *5*). The third marker, rs11209026 ($P = 5.05 \times 10^{-9}$, corrected $P = 1.56 \times 10^{-3}$), is a nonsynonymous SNP (c.1142G>A, p.Arg381Gln) in the *IL23R* gene (GenBank accession: NM_144701, GeneID: 149233) on chromosome 1p31. This gene encodes a subunit of the receptor for the proinflammatory cytokine, interleukin-23 (IL-23), and is therefore an intriguing functional candidate. In addition to Arg381Gln, nine other markers in *IL23R* and in the intergenic region between *IL23R* and the adjacent IL-12 receptor, beta-2 gene (*IL12RB2*), had association *P*-values < 0.0001 in the non-Jewish, ileal CD case-control cohort (Table 1 and table S1a).

We next tested for association of *IL23R* markers in an independent ileal CD case-control cohort, consisting of 401 patients and 433 controls, all of Jewish ancestry (*8*). Significant associations were observed for several of the same markers that were associated in the non-Jewish cohort (Table 1 and table S1b). In a combined analysis of the data from the two ileal CD case-control cohorts (*8*), nine markers had highly significant association *P*-values ranging from 1.60×10^{-9} to 3.36×10^{-13} (Table 1 and table S1b).

We then extended the replication study by performing family-based association testing of 27 *IL23R* region markers in an independent cohort of 883 nuclear families in which both par-

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ents and their IBD (CD, UC, or indeterminate IBD)–affected offspring were available for genotyping (Table 2 and table S2) (*8*). For Arg381Gln and other *IL23R* markers, we observed significant departure from random allele transmission to CD-affected offspring in both non-Jewish and Jewish families, providing further evidence for association between CD and *IL23R*. We also observed distortion of allele transmission to non-Jewish, UC-affected offspring, providing evidence for association of *IL23R* with non-Jewish UC. There was no evidence for association of Arg381Gln or other *IL23R* region markers in the Jewish UC families. In a combined analysis of the data from all 883 nuclear families and both case-control cohorts (*8*), all 10 *IL23R* markers in Table 2 showed highly significant association with IBD, with *P*-values ranging from 3.55×10^{-9} to 6.62×10^{-19}.

The *IL23R* gene is contained within two large blocks of linkage disequilibrium, and markers in the centromeric block containing exons 5 to 11 and part of the intergenic region between *IL23R* and *IL12RB2* have the strongest association signals (Fig. 1). There is no significant association within *IL12RB2* (Fig. 1), and we did not identify a *IL12RB2* SNP in the International HapMap CEU data that is correlated with an IBD-associated, *IL23R* region variant (*8*).

The IL23R protein contains an extracellular domain (composed of a signal sequence, an N-terminal immunoglobulin-like domain, and two cytokine receptor domains), a single transmembrane domain, and a 252–amino acid cytoplasmic domain (*9*). Arg-381, in the cytoplasmic domain, is the fifth amino acid internal to the transmembrane domain and is highly conserved between species (fig. S1). In contrast, two other nonsynonymous *IL23R* SNPs, rs1884444 (His3Gln) and rs7530511 (Pro310Leu), which are located within the extracellular domain, show no evidence for disease association (table S1, a and b).

The glutamine allele of Arg381Gln is much less common than the arginine allele, with an allelic frequency of 1.9% in the non-Jewish patients with ileal CD and 7.0% in non-Jewish controls. The glutamine allele appears to protect against development of CD in both non-Jewish [odds ratio (OR) = 0.26, 95% confidence interval (CI) (0.15 to 0.43)] and Jewish [OR = 0.45, 95% CI (0.27 to 0.73)] case-control cohorts. The glutamine allele is also significantly undertransmitted from heterozygous parents to non-Jewish and Jewish CD-affected offspring, non-Jewish UC-affected offspring, and all IBD-affected offspring (transmitted:nontransmitted = 45:130, $P = 1.32 \times 10^{-10}$ for the IBD phenotype in all 883 families) (Table 2 and table S2). Our discovery of an uncommon protective allele, or conversely, a very common predisposing allele, reflects a major theme in complex genetics; namely, that functional genetic variation exerts a continuum of susceptibility, neutral, and protective effects. Furthermore, alleles conferring protection against one disease may result in increased risk for another (*10*).

Table 1. Non-Jewish and Jewish ileal Crohn's disease (CD) case-control association study results for IL23R region markers with *P*-values < 0.0001 in the non-Jewish cohort. Minor allele frequencies (MAF), allelic test *P*-values, and odds ratios (OR) with 95% confidence intervals (CI) are shown for each case-control cohort (*8*). The ORs shown are for the minor allele. Combined Cochran-Mantel-Haenszel *P*-values are also shown (*8*). UTR, untranslated region.

Marker	Location	Non-Jewish case-control cohort				Jewish case-control cohort				Combined *P*-value
		CD (*n* = 547) MAF	Control (*n* = 548) MAF	*P*-value	OR [95% CI]	CD (*n* = 401) MAF	Control (*n* = 433) MAF	*P*-value	OR [95% CI]	
rs1004819	Intron	0.374	0.280	3.79×10^{-6}	1.53 [1.27,1.84]	0.426	0.334	1.00×10^{-4}	1.48 [1.21,1.82]	1.54×10^{-9}
rs7517847	Intron	0.331	0.443	1.09×10^{-7}	0.62 [0.52,0.74]	0.240	0.352	5.84×10^{-7}	0.58 [0.47,0.72]	3.36×10^{-13}
rs10489629	Intron	0.378	0.475	4.27×10^{-6}	0.67 [0.56,0.80]	0.355	0.465	5.79×10^{-6}	0.63 [0.52,0.77]	1.14×10^{-10}
rs2201841	Intron	0.385	0.291	4.57×10^{-6}	1.52 [1.27,1.83]	0.414	0.315	2.92×10^{-5}	1.53 [1.25,1.89]	5.46×10^{-10}
rs11465804	Intron	0.020	0.063	7.52×10^{-7}	0.30 [0.18,0.51]	0.048	0.096	1.39×10^{-4}	0.47 [0.31,0.71]	5.97×10^{-10}
rs11209026	Arg381Gln	0.019	0.070	5.05×10^{-9}	0.26 [0.15,0.43]	0.033	0.070	7.95×10^{-4}	0.45 [0.27,0.73]	3.55×10^{-11}
rs1343151	Intron	0.275	0.370	2.26×10^{-6}	0.65 [0.54,0.78]	0.229	0.336	1.69×10^{-6}	0.59 [0.47,0.73]	1.64×10^{-11}
rs10889677	Exon-3'UTR	0.385	0.288	1.82×10^{-6}	1.55 [1.29,1.86]	0.419	0.316	1.51×10^{-5}	1.56 [1.27,1.91]	9.58×10^{-11}
rs11209032	Intergenic	0.393	0.293	1.03×10^{-6}	1.56 [1.30,1.87]	0.382	0.298	3.49×10^{-4}	1.45 [1.18,1.79]	1.60×10^{-9}
rs1495965	Intergenic	0.498	0.412	2.93×10^{-5}	1.44 [1.21,1.71]	0.469	0.412	2.04×10^{-2}	1.26 [1.03,1.53]	2.55×10^{-6}

Table 2. Family-based and combined (case-control and family-based) association results. Family-based association *P*-values were computed using the empirical variance estimator implemented in the FBAT software package (*8*). Combined Fisher *P*-values for all case-control (Table 1) and nuclear family cohorts are also shown (*8*). UTR, untranslated region.

Marker	Location	Non-Jewish CD (518 families, 651 affected offspring)	Non-Jewish UC (215 families, 251 affected offspring)	Jewish CD (77 families, 99 affected offspring)	Jewish UC (80 families, 91 affected offspring)	All IBD (883 families, 1,119 affected offspring)	Combined (family-based and case-control) *P*-value
		P-value	*P*-value	*P*-value	*P*-value	*P*-value	
rs1004819	Intron	3.60×10^{-5}	1.20×10^{-3}	1.24×10^{-2}	5.47×10^{-1}	6.06×10^{-8}	1.78×10^{-14}
rs7517847	Intron	2.30×10^{-5}	2.71×10^{-1}	3.50×10^{-2}	5.00×10^{-1}	1.80×10^{-5}	9.99×10^{-16}
rs10489629	Intron	1.87×10^{-3}	2.70×10^{-1}	4.33×10^{-1}	8.21×10^{-1}	1.27×10^{-3}	1.62×10^{-11}
rs2201841	Intron	5.80×10^{-4}	3.21×10^{-4}	3.50×10^{-2}	5.69×10^{-1}	1.04×10^{-7}	1.10×10^{-14}
rs11465804	Intron	1.32×10^{-4}	2.70×10^{-3}	8.90×10^{-5}	3.71×10^{-1}	3.46×10^{-9}	3.33×10^{-16}
rs11209026	Arg381Gln	8.00×10^{-6}	2.97×10^{-4}	9.41×10^{-4}	4.91×10^{-1}	1.32×10^{-10}	6.62×10^{-19}
rs1343151	Intron	9.63×10^{-2}	8.51×10^{-2}	3.30×10^{-2}	1.89×10^{-1}	1.24×10^{-3}	2.74×10^{-12}
rs10889677	Exon-3'UTR	2.60×10^{-3}	3.35×10^{-4}	5.88×10^{-2}	7.32×10^{-1}	1.65×10^{-6}	3.40×10^{-14}
rs11209032	Intergenic	2.68×10^{-3}	3.57×10^{-4}	3.48×10^{-2}	7.50×10^{-1}	2.41×10^{-6}	5.50×10^{-13}
rs1495965	Intergenic	4.07×10^{-4}	1.74×10^{-2}	3.93×10^{-2}	9.21×10^{-1}	1.72×10^{-5}	3.55×10^{-9}

In addition to Arg381Gln, we found several other variants within the *IL23R* gene that are also associated with IBD (Tables 1 and 2 and tables S1 and S2). Marker rs11465804 is an intronic variant in a nonconserved region and is in significant linkage disequilibrium with Arg381Gln (correlation coefficient $r^2 = 0.84$ in the case-control data) and therefore is unlikely to confer disease risk independent of the latter. However, other markers show evidence for association that appears to be independent of Arg381Gln. For example, rs7517847, which has the most significant association *P*-value (3.36×10^{-13}) in the combined analysis of both ileal CD case-control cohorts (Table 1), is not in significant linkage disequilibrium with Arg381Gln ($r^2 = 0.03$ in the case-control data). To identify variants that are independent of the Arg381Gln signal, we performed conditional association testing of the combined case-control data by stratifying on the Arg381Gln genotypes. The *P*-values for these conditional tests (table S3) demonstrate multiple residual association signals throughout *IL23R*, indicating that there are multiple risk variants in the region. The *IL23R* gene is expressed as at least six alternatively spliced mRNAs, which generate diverse isoforms of the receptor protein (*11*). The most common splice variants result in the deletion of exons 7 and/or 10. We therefore speculate that the multiple genetic association signals detected in the centromeric portion of *IL23R* (Fig. 1) could exert their influence via differential splicing.

Notably, we found no evidence for association in our non-Jewish, ileal CD case-control cohort (table S4) with the *IL12RB1* gene, which encodes the second subunit of the IL-23 receptor (*9*), or the *IL23A* and *IL12B* genes, which encode the p19 and p40 subunits, respectively, of the heterodimeric IL-23 cytokine (*12*).

Previous work with mouse models has documented a requirement for IL-23 in murine colitis (*13*), experimental autoimmune encephalitis (*14*), and collagen-induced arthritis (*15*). IL-23 activity is present in the terminal ileum (*16*) and colon (*17*), and the present study demonstrates that *IL23R* variants are associated with both small intestinal (ileal CD) and large intestinal (UC) inflammation. Furthermore, transgenic expression of IL-23 subunit p19 results in severe systemic inflammation, including in the small and large intestine (*18*), highlighting this pathway's particular role in promoting strong activation of effector T cells and perpetuation of organ-specific inflammatory responses. At least part of this effect is likely mediated via inflammatory, IL-17–producing T cells (*19–23*), and elevated IL-17 levels have been observed in the colonic mucosa of both CD and UC patients (*24*).

Taken together, these findings suggest that blockade of the IL-23 signaling pathway would be a rational therapeutic strategy for IBD. In support of this, a monoclonal antibody directed against the p40 subunit of the receptor, which blocks both IL-23 and IL-12 proinflammatory activities, has produced promising results in a clinical trial of Crohn's disease (*25*). It has been postulated that specific targeting of the IL23p19/IL23R pathway may be particularly effective in blocking organ-specific inflammation, with less compromise of protective responses (*26*). However, at least one model of murine colitis is worsened in the absence of IL-23, implicating a role for IL-23 in the down-regulation of IL-12 (*27*). In addition, IL-23 function may be important for proper responses to mycobacterial (*28*, *29*) and intestinal infections (*22*). In assessing therapeutic approaches, the strong protective effect of the Arg381Gln allele could potentially be exploited to define desired functional outcomes (*10*). The contribution of the *IL23R* pathway to IBD will likely involve more than simple gain- or loss-of-function *IL23R* variants, and therapeutic interventions will be improved by a better understanding of the context and tissue-specific events associated with functional *IL23R* polymorphisms.

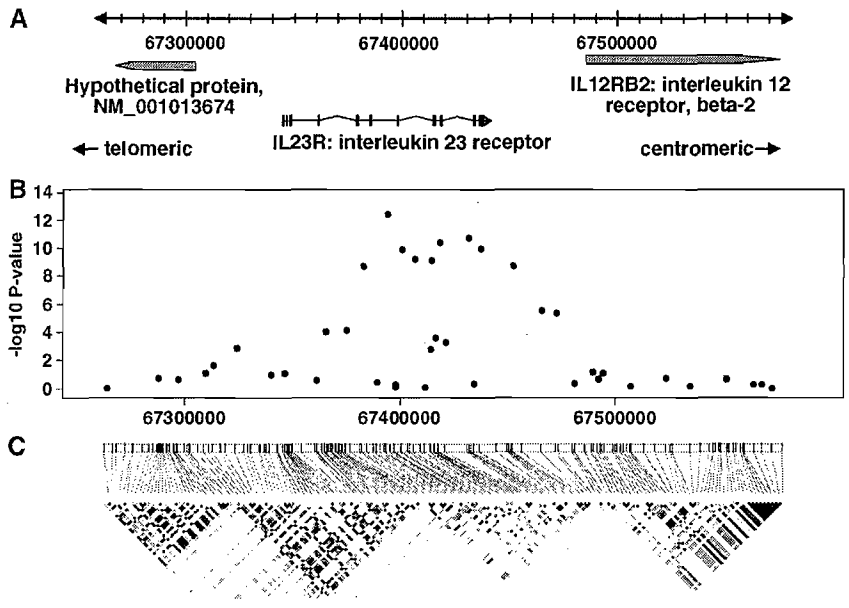

Fig. 1. Association signals in the *IL23R* gene region on chromosome 1p31. (**A**) Genomic locations of genes on chromosome 1p31 between 67,260,000 and 67,580,000 base pairs (Build 35). (**B**) The negative $\log_{10}$ association *P*-values (Cochran-Mantel-Haenszel chi-square test) from the combined Jewish and non-Jewish case-control cohorts are plotted for genotyped markers in the region. (**C**) Pairwise r^2 plot for International HapMap CEU data. The intensity of the shading is proportional to r^2. The *IL23R* gene is contained within two blocks of linkage disequilibrium, and the association signals are strongest in the centromeric block, which contains exons 5 to 11 and extends into the intergenic region between *IL23R* and *IL12RB2*. Note that markers in the block encompassing the *IL12RB2* gene do not demonstrate significant association.

References and Notes

1. D. K. Podolsky, *N. Engl. J. Med.* **347**, 417 (2002).
2. E. V. Loftus Jr., *Gastroenterology* **126**, 1504 (2004).
3. S. Vermeire, P. Rutgeerts, *Genes Immun.* **6**, 637 (2005).
4. J.-P. Hugot *et al.*, *Nature* **411**, 599 (2001).
5. Y. Ogura *et al.*, *Nature* **411**, 603 (2001).
6. J. D. Rioux *et al.*, *Nat. Genet.* **29**, 223 (2001).
7. V. D. Peltekova *et al.*, *Not. Genet.* **36**, 471 (2004).
8. Materials and methods are available as supporting material on *Science* Online.
9. C. Parham *et al.*, *J. Immunol.* **168**, 5699 (2002).
10. J. H. Nadeau, E. J. Topol, *Nat. Genet.* **38**, 1095 (2006).
11. X. Y. Zhang *et al.*, *Immunogenetics* **57**, 934 (2006).
12. B. Oppmann *et al.*, *Immunity* **13**, 715 (2000).
13. D. Yen *et al.*, *J. Clin. Invest.* **116**, 1310 (2006).
14. D. J. Cua *et al.*, *Noture* **421**, 744 (2003).
15. C. A. Murphy *et al.*, *J. Exp. Med.* **198**, 1951 (2003).
16. C. Becker *et al.*, *J. Clin. Invest.* **112**, 693 (2003).
17. I. J. Fuss *et al.*, *Inflamm. Bowel Dis.* **12**, 9 (2006).
18. M. T. Wiekowski *et al.*, *J. Immunol.* **166**, 7563 (2001).
19. S. Aggarwal, N. Ghilardi, M. H. Xie, F. J. de Sauvage, A. L. Gurney, *J. Biol. Chem.* **278**, 1910 (2003).
20. C. L. Langrish *et al.*, *J. Exp. Med.* **201**, 233 (2005).
21. E. Bettelli *et al.*, *Nature* **441**, 235 (2006).
22. P. R. Mangan *et al.*, *Nature* **441**, 231 (2006).
23. M. Veldhoen, R. J. Hocking, C. J. Atkins, R. M. Locksley, B. Stockinger, *Immunity* **24**, 179 (2006).
24. S. Fujino *et al.*, *Gut* **52**, 65 (2003).
25. P. J. Mannon *et al.*, *N. Engl. J. Med.* **351**, 2069 (2004).
26. B. S. McKenzie, R. A. Kastelein, D. J. Cua, *Trends Immunol.* **27**, 17 (2006).
27. C. Becker *et al.*, *J. Immunol.* **177**, 2760 (2006).
28. F. A. Verreck *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 4560 (2004).
29. A. M. Cooper *et al.*, *J. Immunol.* **168**, 1322 (2002).

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Supporting Online Material

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Materials and Methods
Fig. S1
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